

#8

1652

RAW SEQUENCE LISTING

DATE: 08/01/2001

PATENT APPLICATION: US/09/654,652A

TIME: 13:55:40

Input Set : A:\4910-8.app

Output Set: N:\CRF3\08012001\I654652A.raw

P.S.

3 <110> APPLICANT: SHYUR, LIE-FEN
 4 CHEN, JUI-LIN
 5 YANG, NING-SUN
 7 <120> TITLE OF INVENTION: A TRUNCATED FORM OF FIBROBACTER SUCCINOGENES 1, 3-1,
 8 4-BETA-D-GLUCANASE WITH IMPROVED ENZYMATIC ACTIVITY AND
 9 THERMO-TOLERANCE
 11 <130> FILE REFERENCE: 4910-8
 13 <140> CURRENT APPLICATION NUMBER: 09/654,652A
 14 <141> CURRENT FILING DATE: 2000-09-05
 16 <160> NUMBER OF SEQ ID NOS: 11
 18 <170> SOFTWARE: PatentIn Ver. 2.1
 20 <210> SEQ ID NO: 1
 21 <211> LENGTH: 248
 22 <212> TYPE: PRT
 23 <213> ORGANISM: Artificial Sequence
 25 <220> FEATURE:
 26 <223> OTHER INFORMATION: Description of Artificial Sequence: Modified enzyme
 27 with enhanced activity and thermal stability
 29 <400> SEQUENCE: 1
 30 Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
 31 1 5 10 15
 33 Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala
 34 20 25 30
 36 Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile
 37 35 40 45
 39 Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys
 40 50 55 60
 42 Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala
 43 65 70 75 80
 45 Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln
 46 85 90 95
 48 Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp
 49 100 105 110
 51 Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser
 52 115 120 125
 54 Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu
 55 130 135 140
 57 Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
 58 145 150 155 160
 60 Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly
 61 165 170 175
 63 Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr
 64 180 185 190
 66 Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn
 67 195 200 205
 69 Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu
 70 210 215 220

ENTERED

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72 Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val
73 225                230                235                240
75 Pro Arg Asp Asp Glu Pro Ala Pro
76                245
79 <210> SEQ ID NO: 2
80 <211> LENGTH: 267
81 <212> TYPE: PRT
82 <213> ORGANISM: Artificial Sequence
84 <220> FEATURE:
85 <223> OTHER INFORMATION: Description of Artificial Sequence: Modified enzyme
86     with enhanced activity and thermal stability
88 <400> SEQUENCE: 2
89 Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
90  1      5      10      15
92 Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala
93      20      25      30
95 Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile
96      35      40      45
98 Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys
99      50      55      60
101 Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala
102 65      70      75      80
104 Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln
105      85      90      95
107 Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp
108      100     105     110
110 Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser
111     115     120     125
113 Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu
114     130     135     140
116 Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
117 145     150     155     160
119 Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly
120     165     170     175
122 Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr
123     180     185     190
125 Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn
126     195     200     205
128 Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu
129     210     215     220
131 Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val
132 225     230     235     240
134 Pro Arg Asp Asp Glu Pro Ala Pro Asn Ser Ser Ser Val Asp Lys Leu
135     245     250     255
137 Ala Ala Ala Leu Glu His His His His His His
138     260     265
141 <210> SEQ ID NO: 3
142 <211> LENGTH: 349
143 <212> TYPE: PRT

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144 <213> ORGANISM: Fibrobacter succinogenes
146 <400> SEQUENCE: 3
147 Met Asn Ile Lys Lys Thr Ala Val Lys Ser Ala Leu Ala Val Ala Ala
148   1           5           10           15
150 Ala Ala Ala Ala Leu Thr Thr Asn Val Ser Ala Lys Asp Phe Ser Gly
151           20           25           30
153 Ala Glu Leu Tyr Thr Leu Glu Glu Val Gln Tyr Gly Lys Phe Glu Ala
154           35           40           45
156 Arg Met Lys Met Ala Ala Ala Ser Gly Thr Val Ser Ser Met Phe Leu
157           50           55           60
159 Tyr Gln Asn Gly Ser Glu Ile Ala Asp Gly Arg Pro Trp Val Glu Val
160  65           70           75           80
162 Asp Ile Glu Val Leu Gly Lys Asn Pro Gly Ser Phe Gln Ser Asn Ile
163           85           90           95
165 Ile Thr Gly Lys Ala Gly Ala Gln Lys Thr Ser Glu Lys His His Ala
166           100          105          110
168 Val Ser Pro Ala Ala Asp Gln Ala Phe His Thr Tyr Gly Leu Glu Trp
169           115          120          125
171 Thr Pro Asn Tyr Val Arg Trp Thr Val Asp Gly Gln Glu Val Arg Lys
172           130          135          140
174 Thr Glu Gly Gly Gln Val Ser Asn Leu Thr Gly Thr Gln Gly Leu Arg
175 145           150           155           160
177 Phe Asn Leu Trp Ser Ser Glu Ser Ala Ala Trp Val Gly Gln Phe Asp
178           165           170           175
180 Glu Ser Lys Leu Pro Leu Phe Gln Phe Ile Asn Trp Val Lys Val Tyr
181           180           185           190
183 Lys Tyr Thr Pro Gly Gln Gly Glu Gly Gly Ser Asp Phe Thr Leu Asp
184           195           200           205
186 Trp Thr Asp Asn Phe Asp Thr Phe Asp Gly Ser Arg Trp Gly Lys Gly
187           210           215           220
189 Asp Trp Thr Phe Asp Gly Asn Arg Val Asp Leu Thr Asp Lys Asn Ile
190 225           230           235           240
192 Tyr Ser Arg Asp Gly Met Leu Ile Leu Ala Leu Thr Arg Lys Gly Gln
193           245           250           255
195 Glu Ser Phe Asn Gly Gln Val Pro Arg Asp Asp Glu Pro Ala Pro Gln
196           260           265           270
198 Ser Ser Ser Ser Ala Pro Ala Ser Ser Ser Ser Val Pro Ala Ser Ser
199           275           280           285
201 Ser Ser Val Pro Ala Ser Ser Ser Ser Ala Phe Val Pro Pro Ser Ser
202           290           295           300
204 Ser Ser Ala Thr Asn Ala Ile His Gly Met Arg Thr Thr Pro Ala Val
205 305           310           315           320
207 Ala Lys Glu His Arg Asn Leu Val Asn Ala Lys Gly Ala Lys Val Asn
208           325           330           335
210 Pro Asn Gly His Lys Arg Tyr Arg Val Asn Phe Glu His
211           340           345
214 <210> SEQ ID NO: 4
215 <211> LENGTH: 744
216 <212> TYPE: DNA

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Output Set: N:\CRF3\08012001\I654652A.raw

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217 <213> ORGANISM: Artificial Sequence
219 <220> FEATURE:
220 <223> OTHER INFORMATION: Description of Artificial Sequence: DNA encoding a
221     modified enzyme
223 <220> FEATURE:
224 <221> NAME/KEY: CDS
225 <222> LOCATION: (1)..(744)
227 <400> SEQUENCE: 4
228 atg gtt agc gca aag gat ttt agc ggt gcc gaa ctc tac acg tta gaa 48
229 Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
230 1 5 10 15
232 gaa gtt cag tac ggt aag ttt gaa gcc cgt atg aag atg gca gcc gca 96
233 Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala
234 20 25 30
236 tcg gga aca gtc agt tcc atg ttc ctc tac cag aat ggt tcc gaa atc 144
237 Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile
238 35 40 45
240 gcc gat gga agg ccc tgg gta gaa gtg gat att gaa gtt ctc ggc aag 192
241 Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys
242 50 55 60
244 aat ccg ggc agt ttc cag tcc aac atc att acc ggt aag gcc ggc gca 240
245 Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala
246 65 70 75 80
248 caa aag act agc gaa aag cac cat gct gtt agc ccc gcc gcc gat cag 288
249 Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln
250 85 90 95
252 gct ttc cac acc tac ggt ctc gaa tgg act ccg aat tac gtc cgc tgg 336
253 Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp
254 100 105 110
256 act gtt gac ggt cag gaa gtc cgc aag acg gaa ggt ggc cag gtt tcc 384
257 Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser
258 115 120 125
260 aac ttg aca ggt aca cag gga ctc cgt ttt aac ctt tgg tcg tct gag 432
261 Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu
262 130 135 140
264 agt gcg gct tgg gtt ggc cag ttc gat gaa tca aag ctt ccg ctt ttc 480
265 Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
266 145 150 155 160
268 cag ttc atc aac tgg gtc aag gtt tat aag tat acg ccg ggc cag ggc 528
269 Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly
270 165 170 175
272 gaa ggc ggc agc gac ttt acg ctt gac tgg acc gac aat ttt gac acg 576
273 Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr
274 180 185 190
276 ttt gat ggc tcc cgc tgg ggc aag ggt gac tgg aca ttt gac ggt aac 624
277 Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn
278 195 200 205
280 cgt gtc gac ctc acc gac aag aac atc tac tcc aga gat ggc atg ttg 672
281 Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu

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Input Set : A:\4910-8.app

Output Set: N:\CRF3\08012001\I654652A.raw

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282      210      215      220
284 atc ctc gcc ctc acc cgc aaa ggt cag gaa agc ttc aac ggc cag gtt 720
285 ile leu ala leu thr arg lys gly gln glu ser phe asn gly gln val
286 225      230      235      240
288 ccg aga gat gac gaa cct gct ccg 744
289 pro arg asp asp glu pro ala pro
290      245
293 <210> SEQ ID NO: 5
294 <211> LENGTH: 804
295 <212> TYPE: DNA
296 <213> ORGANISM: Artificial Sequence
298 <220> FEATURE:
299 <223> OTHER INFORMATION: Description of Artificial Sequence: DNA encoding a
300 modified enzyme
302 <220> FEATURE:
303 <221> NAME/KEY: CDS
304 <222> LOCATION: (1)..(804)
306 <400> SEQUENCE: 5
307 atg gtt agc gca aag gat ttt agc ggt gcc gaa ctc tac acg tta gaa 48
308 Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
309 1 5 10 15
311 gaa gtt cag tac ggt aag ttt gaa gcc cgt atg aag atg gca gcc gca 96
312 Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala
313 20 25 30
315 tcg gga aca gtc agt tcc atg ttc ctc tac cag aat ggt tcc gaa atc 144
316 Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile
317 35 40 45
319 gcc gat gga agg ccc tgg gta gaa gtg gat att gaa gtt ctc ggc aag 192
320 Ala Asp Gly Arg Pro Trp Val Glu Val Asp ile Glu Val Leu Gly Lys
321 50 55 60
323 aat ccg ggc agt ttc cag tcc aac atc att acc ggt aag gcc ggc gca 240
324 Asn Pro Gly Ser Phe Gln Ser Asn ile ile Thr Gly Lys Ala Gly Ala
325 65 70 75 80
327 caa aag act agc gaa aag cac cat gct gtt agc ccc gcc gcc gat cag 288
328 Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln
329 85 90 95
331 gct ttc cac acc tac ggt ctc gaa tgg act ccg aat tac gtc cgc tgg 336
332 Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp
333 100 105 110
335 act gtt gac ggt cag gaa gtc cgc aag acg gaa ggt ggc cag gtt tcc 384
336 Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser
337 115 120 125
339 aac ttg aca ggt aca cag gga ctc cgt ttt aac ctt tgg tcg tct gag 432
340 Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu
341 130 135 140
343 agt gcg gct tgg gtt ggc cag ttc gat gaa tca aag ctt ccg ctt ttc 480
344 Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
345 145 150 155 160
347 cag ttc atc aac tgg gtc aag gtt tat aag tat acg ccg ggc cag ggc 528

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→ Use of n and/or Xaa has been detected in the Sequence Listing.

Review the Sequence Listing to insure a corresponding explanation is presented in the <220> to <223> fields of each sequence using n or Xaa.

VERIFICATION SUMMARY

DATE: 08/01/2001

PATENT APPLICATION: US/09/654,652A

TIME: 13:55:41

Input Set : A:\4910-8.app

Output Set: N:\CRF3\08012001\I654652A.raw

L:539 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:11